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Prevalence of plasmid-mediated *qnr* determinants and gyrase alteration in *Klebsiella pneumoniae* isolated from a university teaching hospital in Malaysia

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Abstract. – BACKGROUND AND OBJECTIVES: The ciprofloxacin resistance of *Klebsiella (K.) pneumoniae* is mediated primarily through alterations in type II topoisomerase *(gyrA)* gene and plasmid-mediated quinolone resistance-conferring genes *(qnr)*.

This study aimed to define the prevalence of plasmid-mediated quinolone resistance-conferring genes *(qnr)* and type II topoisomerase *(gyrA)* alterations of a population of ciprofloxacin-resistant (n = 21), intermediate (n = 8), and sensitive (n = 18) *K. pneumoniae* isolates obtained from a teaching hospital at Kuala Lumpur, Malaysia.

MATERIALS AND METHODS: A multiplex PCR assay was performed for simultaneous detection of *qnrA, qnrB* and *qnrS*. Sequence analysis of the amplified *gyrA* and *gyrB* regions of the isolates were performed.

RESULTS: The findings in this study revealed the emergence of a high prevalence (48.9%) of *qnr* determinants in our isolates. Four variants of plasmid-mediated qnr determinants (*qnrB1*, *qnrB6*, *qnrB10* and *qnrS1*) were detected from 11 (52.4%) ciprofloxacin-resistant, 5 (62.5%) intermediate and 7 (38.9%) sensitive isolates. *gyrA* alterations were detected from 18 (85.7%) ciprofloxacin-resistant isolates. Single *gyrA* alterations, Ser83→Tyr, Ser83→Ile, and Asp87→Gly, and double alterations, Ser83→Phe plus Asp87→Ala and Ser83→Tyr plus Asp87→Asn were detected. While ciprofloxacin resistance was significantly associated with *gyrA* alteration (Ser83, $p = 0.003$; Asp87, $p = 0.005$; double alteration, $p = 0.016$), no significant association of ciprofloxacin resistance was noted with the presence of *qnr* determinants ($p = 0.283$).

CONCLUSIONS: The findings in this study demonstrate the emergence of *qnr* determinants and *gyrA* alterations contributed to the development and spread of fluoroquinolone resistance in the Malaysian isolates.

Key Words:
Klebsiella pneumoniae, Gyrase A, Plasmid-mediated *qnr* determinants, Ciprofloxacin.

Introduction

Klebsiella (K.) pneumoniae is a frequent cause of urinary tract infections, nosocomial pneumonia, and intra-abdominal infections amongst hospitalized patients. In recent years, emergence of ciprofloxacin-resistant *K. pneumoniae* isolates has been widely reported in Europe, North America and Asia[1], along with the upsurge of resistance to other antibiotics. The ciprofloxacin resistance of *K. pneumoniae* is mediated primarily through alterations in type II topoisomerase (*gyrA*) gene or changes in the drug entry and efflux[2,3]. More recently, low-level resistance to quinolones conferred by plasmid-mediated quinolone resistance-conferring genes (*qnr*) has been reported[1]. *qnr* genes protects DNA gyrase and topoisomerase from inhibition by quinolones and have been often associated with genes that confer resistance to other classes of antibiotics (e.g. β-lactams and aminoglycosides). In addition, isolates with *qnr* resistance may represent a serious concern in the clinical environment as it can contribute to the rapid development and spread of fluoroquinolone resistance.

Limited information is available on the ciprofloxacin-resistance mechanisms of *K. pneumoniae* isolates in the Southeast Asia region, including Malaysia. The aim of this study was to define the prevalence of plasmid-mediated *qnr* resistance and *gyrA* alteration of a population of ciprofloxacin-resistant (n = 21), intermediate (n = 8), and sensitive (n = 18) *K. pneumoniae* clinical isolates from University Malaya Medical Center (UMMC), a Teaching Hospital at Kuala Lumpur, Malaysia.

Materials and Methods

Klebsiella Clinical Isolates

These were multi-drug resistant isolates obtained from various types of clinical specimens in

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Table I. Detection of plasmid-mediated *qnr* genes in *K. pneumoniae* isolates.

Ciprofloxacin susceptibility	No. (%) strain with *qnr* determinants				
	qnrB1	*qnrB6*	*qnrB10*	*qnrS1*	Total
Sensitive (n = 18)	2 (11.1)	0 (0)	1 (5.6)	4 (22.2)	7 (38.9)
Intermediate (n = 8)	2 (25.0)	1 (12.5)	2 (25.0)	0 (0)	5 (62.5)
Resistant (n = 21)	4 (19.0)	1 (4.8)	2 (9.5)	4 (19.0)	11 (52.4)
Total (n = 47)	8 (17.0)	2 (4.3)	5 (10.6)	8 (17.0)	23 (48.9)

the Microbiology Diagnostic Laboratory from 2006-2008. The identity of the isolates was confirmed based on the sequence analysis of the internal transcribed spacer gene region, as described by Wang et al[4]. Minimum inhibitory concentrations (MICs) of the isolates to ciprofloxacin were confirmed using E tests strips (bioMéreiux SA, Marcy L'Etoile, France) according to the manufacturer's instructions. Ciprofloxacin resistance was defined as minimum inhibitory concentration (MIC) ≥ 4 µg/ml, intermediate as MIC = 2 µg/ml and sensitive as MIC ≤1 µg/ml[5].

Amplification and Sequence Analysis of Resistance Genes

A multiplex polymerase chain reaction (PCR) assay was performed for the simultaneous detection of *qnrA, qnrB* and *qnrS* as described by Cattoir et al[6], using boiled bacterial extracts as DNA templates. The *gyrA* and *gyrB* regions of the isolates were amplified as described by Ling et al[7]. The amplicons were purified using GeneAll (PCR SV PCR kit, Seoul, Korea) and the subsequent sequencing reaction was performed with the Big Dye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on an ABI-377 genetic Analyzer (Applied Biosystems, Lincoln Centre Drive, Foster City, CA, USA). The *qnr* sequences were subjected to BLAST search for matching sequences in the GenBank database. The gyrase nucleotide sequences and the deduced amino acids were compared with that of *K. pneumoniae* ATCC (American Type Culture Collection) 13883 (GenBank accession number DQ673325) using ClustalW alignment program.

Statistical Analysis

The association of each resistance mechanism for ciprofloxacin-resistance was analysed using the Mann-Whitney test of the SPSS statistical package (version 13.0) (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered statistically significant.

Results

The ciprofloxacin MICs of our isolates ranged from 0.25 µg/ml to ≥ 32 µg/ml, with MIC_{50} and MIC_{90} being 2 µg/ml and ≥ 32 µg/ml, respectively. The ciprofloxacin MICs for resistant isolates were as follows: 4 µg/ml, 2 isolates (9.5%); 8

Table II. Alterations in *gyrA* gene regions of *K. pneumoniae* isolates.

Ciprofloxacin susceptibility	No.(%) isolate with *gyrA* alterations							
			codon Ser83 (TCC)			codon Asp87 (GAC)		
	Single alteration	Double alteration	Tyr (TAC)	Ile (ATC)	Phe (TTC)	Gly (GGC)	Ala (GCC)	Asn (AAC)
Sensitive (n =18)	5 (27.8)	0 (0)	5 (27.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate (n = 8)	5 (62.5)	0 (0)	4 (50)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)
Resistant (n = 21)	10 (47.6)	8 (38.1)	6 (28.6)	6 (28.6)	5 (23.8)	1 (4.8)	5 (23.8)	3 (14.3)
Total (n = 47)	20 (42.6)	8 (17.0)	15 (31.9)	6 (12.8)	5 (10.6)	2 (4.3)	5 (10.6)	3 (6.4)

Ser, serine; Asp, Aspartic acid; Tyr, tyrosine; Ile, isoleucine; Phe, phenylalanine; Gly, glycine; Ala, alanine; Asn, asparagine.

µg/ml, 1 isolate (4.8%); 12 µg/ml, 3 isolates (14.3%); and ≥ 32 µg/ml, 15 isolates (71.4%).

The prevalence and diversity of *qnr* determinants of our isolates are shown in Table I. Four *qnr* determinants (*qnrB1, qnrB6, qnrB10* and *qnrS1*) were identified; with *qnrB* detected more frequently than *qnrS*. No *qnrA* was detected from our isolates. *qnr* determinants were detected in 11 (52.4%) of the ciprofloxacin-resistant isolates, as well as 7 (38.9%) and 5 (62.5%) sensitive and intermediate isolates, respectively. No significant association of ciprofloxacin resistance with the presence of *qnr* was noted ($p = 0.283$). Of 23 *qnr* positive isolates, 12 (52.2%) had *gyrA* alterations. Three ciprofloxacin-resistant isolates (MICs of 8, 12, ≥ 32 µg/ml) with *qnr* determinants (*qnrB6, qnrS1, qnrB1)* had no *gyrA* alteration.

Alterations in the *gyrA* region, as indicated in Table II, were detected in 28 (59.6%) isolates, of which 20 (42.6%) and 8 (17.0%) had single and double alterations, respectively. The *gyrA* alterations involve codons at the position 83 (Ser) and 87 (Asp). Of the 21 ciprofloxacin-resistant isolates, 10 (47.6%) and 8 (38.1%) had single and double alterations in the *gyrA* region, respectively. Three types of single alterations were noted in the resistant isolates: Ser83→Tyr (3 isolates), Ser83→Ile (5 isolates), and Asp87→Gly (2 isolates). Two types of double alterations were detected in the resistant isolates (all MICs ≥32 µg/ml): Ser83→Phe plus Asp87→Ala (5 isolates), and Ser83→Tyr plus Asp87→Asn (3 isolates). Single alteration (Ser83→Tyr substitution) was the only alteration detected in 5 (27.8%) and 4 (50%) sensitive and intermediate isolates, respectively. When the intermediate isolates were grouped as resistant isolates, significant association of ciprofloxacin resistance was observed with *gyrA* alteration at Ser83 ($p = 0.003$), Asp87 ($p = 0.005$) and double alterations ($p = 0.016$).

Discussion

High prevalences of *qnr* determinants have been reported in many geographical regions[1]. This study reported the emergence of a high prevalence (48.9%) of *qnr* determinants in the Malaysian *K. pneumoniae* isolates. Isolates with *qnr* determinants are known to harbor multiple ciprofloxacin resistance mechanisms including alterations in *gyrA*[8] or decreased drug permeability[9], thus, facilitating high ciprofloxacin resistance. However, acquisition of *qnr* genes does not render a wild-type organism fluoroquinolone insusceptible[1]. This is also observed in this study whereby no significant association of ciprofloxacin resistance was observed with the presence of *qnr* determinants.

All *K. pneumoniae* isolates with double alterations at the *gyrA* region show high ciprofloxacin MICs (≥ 32 µg/ml) in this study. The *gyrA* alterations detected from 18 (85.7%) of 21 ciprofloxacin-resistant isolates in this study are similar to those reported in other geographical regions such as Japan, North America and Europe[2,10,11]. Although *gyrA* alteration (Ser83→Leu) has been described in *K. pneumoniae* isolates from two Asian countries, China[12] and Singapore[13], it was not observed in this study. The alteration in *gyrB* gene has been reported in members of *Enterobacteriaceae* such as *Salmonella*[14] and *Proteus mirabilis*[15]. However; it has not been reported for *K. pneumoniae.* Similarly, no *gyrB* alteration was detected in this study.

Conclusions

These findings demonstrate that the emergence of *qnr* determinants and *gyrA* alterations contributed to the development and spread of fluoroquinolone resistance in the Malaysian isolates. A correct use of antibiotics and continuously monitoring of resistance patterns in *K. pneumoniae* in our hospital settings are necessary.

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Conflict of Interest

None declared.

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